REMARKS

This amendment is being filed with a request for continued examination in the above-identified application. The request for continued examination follows an Advisory Action issued by the Examiner on March 14, 2003. In the advisory action, the Examiner refused to enter an after final amendment filed on March 5, 2003 on the basis that the amendment allegedly would raise new issues, i.e. that the monoclonal antibody activates without being artificially crosslinked with a secondary antibody.

Applicant's invention is directed to a monoclonal antibody which is specific for human CD28 and activates human T-lymphocytes of several to all sub-groups without being artificially crosslinked with a secondary antibody and without occupancy of an antigen receptor of the human T-lymphocytes and thus antigen-non-specific. A review of the specification in the instant application makes it very clear that the features of the antibody which distinguish it over the art are the facts that 1) the antibody does not require crosslinkage with a secondary antibody and 2) there is no occupancy of the antigen receptor on the T-lymphocyte, thus allowing it to be directly activating (see specification at page 2, lines 8-9, page 3 last paragraph, and Example 3 at page 9).

As previously argued, June et al. discloses a method for selectively stimulating proliferation of T cells by stimulating the T cell receptor (TCR)/CD3 complex or the CD2 surface protein. Proliferation is then induced with an accessory molecule, e.g, CD28 or CD9, which binds its cognate ligand. The primary activation signal actually requires the second, costimulatory signal (col. 5, lines 17-20). June discloses that various antibodies to CD28 have been reported including monoclonal antibodies: 9.3, KOLT-2, 15E8, 248.23.2 and EX5.3D10 (col. 5, lines 52-57). Careful review of the scientific literature which details the characteristics of these different antibodies reveals that these antibodies are not direct stimulatory antibodies of T cells.

Monoclonal (Mab) 9.3 is murine IgG2a antibody which is directed against the T cell CD28 receptor. June et al. (1990) (*Immunology Today*, 11:211-216) discloses that mAb9.3 stimulation of the CD28 receptor alone did not induce T-cell proliferation (see page 213, second paragraph) and makes reference to several technical papers which describe the effects of the binding of mAb 9.3 to the T cell. June et al. (1987) *Mol. Cell. Biology*, 7:4472-4481 discloses that "the binding of monoclonal antibodies to the CD28

antigen on purified T cells does not result in proliferation", see e.g. abstract of the paper. Additionally, Hara et al. (1985) *J. Exp. Med.* 161:1513-1524 discloses that "mAb 9.3 was not mitogenic either for PMS or isolated T cells. In the presence of TPA, mAb 9.3 was strongly mitogenic for T cells" (see e.g., p. 1516, third paragraph).

Experimental data from other technical papers further demonstrate the absence of T-cell activation by human T-cells with mAb 9.3 alone. For example in a proliferation time course assaying the stimulatory effects of mAb9.3 in combination with CD2 or CD3 mAb, proliferation was not detected when mAb 9.3 was added alone (see e.g. Figure 2 in Van Lier et al. (1988) *Eur. J. Immunol.*, 8:167-172). Also, T cells cultured in the presence of mAb 9.3 alone did not show any induction of an activation as measured by the increase in IL-2 production or secretion (see e.g. Table III in Verwilghen et al. (1993) *J. Immunol.* 150: 835-846).

Monoclonal (mAb) Kolt-2 is a murine IgG1 antibody which is directed against the T cell CD28 receptor (June et al. (1990), *Immunology Today*, 11:211-216). The Kolt-2 mAb behaved similarly to the mAb 9.3 in T cell mitogenic assays. Moreover, the antibody by itself was not mitogenic but in combination with PMA, T-cell proliferation was observed (see e.g., Van Lier et al. (1988) *Eur. J. Immunol.*, 8:167-172 at page 168). Also, North et al. makes additional disclosure that mAb Kolt-2 does not have a stimulatory effect on the proliferation of purified T cells in normal donors or patients with CVID as measured by ^{3}H-thymidine incorporation (North et al. (1994) *Clin. Exp. Immunol.*, 95: 204-208, Figures 1 and 2). Denning et al. further discloses that "Kolt-2 alone had no effect upon thymocyte proliferation" (Denning et al. (1988) *J. Immunol.* 141: at page 2982, second paragraph).

Monoclonal (mAb) 15E-8 is a murine IgG1 antibody which is directed against the T cell CD28 receptor (June et al. (1990), *Immunology Today*, 11:211-216). Romano et al. discloses that anti-CD28 MAB CLB-CD28/1 clone 15E8 alone does not induce proliferation of PBMC from normal donors as determined from ^{3}H-thymidine incorporation (Romano et al. (1993) *Cellular Immunology*, 148:455-463, Figures 4 and 5). Another paper of Romano discloses that the 15E8 antibody alone does not induce IL-2 production by PBMC from normal donors (see e.g., Romano et al. (1994) *Cellular*

Immunology, 156:371-377 at Table 1). Los et al further discloses that an anti-CD28 mAb, termed LCB28, which is presumably MAB CLB-CD28/1 alone does not induce IL-2 production in human T cells (Los et al. (1995) *EMBO J.* 14:3731-3740).

Monoclonal (mAb) 248.23.2 is a murine IgM antibody which is directed against the T cell CD28 receptor (June et al. (1990), *Immunology Today*, 11:211-216). Pierres et al. provides further experimental evidence that human thymocytes or thymic subsets do not respond to anti-CD28 mAb 243 with proliferation as determined by ^{3}H-thymidine incorporation (Pierres et al. (1990) *J. Immunol.* 144, 1202-1207, see Tables III and IV).

Monoclonal antibody Ex5.3D10 is disclosed in June et al. A search of databases reveals no further disclosure about this antibody. This antibody was not available from the European ATCC as evidenced by the attached email regarding availability of the antibody. Moreover, an on-line inquiry into the US ATCC data base also reveals that the antibody is not available. The ATCC deposit number ((HB11373) recited in the June et al. patent at column 6, lines 7-9) for the EX5.3D10 antibody was searched and no entries were found (see the attached print-out of that search).

Thus, even if the motivation existed to screen the June et al. antibodies, the result would not be to arrive at an antibody of the instant invention which is directly activating and does not require crosslinkage with a secondary antibody. Furthermore, the references cited herein are part of the state of the art and must be considered herein in determining whether the necessary motivation exists. They clearly establish, for the reasons summarized above, that motivation did not exist to screen the June et al. antibodies since the antibodies were known not to be directly stimulating.

In view of the above remarks and amendments, favorable consideration is courteously requested. However, if there are any remaining issues which can be expeditiously resolved by a telephone conference, the Examiner is courteously requested to telephone the undersigned at the number indicated below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

Robert E. McCarthy, Reg. No. 46,044
Representative Capacity

Anthony J. Zelano, Reg. No. 27,969
Attorney for Applicants
MILLEN, WHITE, ZELANO & BRANIGAN, P.C.
Arlington Courthouse Plaza
2200 Clarendon Blvd., Suite 1400
Arlington, VA 22201
Direct Dial: (703) 812-5322
email: mccarthy@mwzb.com

Date filed: April 28, 2003
K:/Albre/20/amendment to accompany RCE.word